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Commercial micropropagation in Germany

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Summary

Since the first experiments of Gottlieb Haberlandt (1854-1945) in the early 1900 on the *in vitro* cultivation of plant tissue, the fields of application have expanded from research of plant physiology to applications in breeding, molecular and microbiology and it became also an important tool for commercial plant production (LAIMER and RÜCKER, 2003). Different fields of application and their perspectives will be discussed. Numbers of commercial micropropagation in Germany will be presented from 2004 to 2017 and possible reasons for changes investigated.

Keywords: *in vitro* culture; plant tissue culture; commercial micropropagation

Introduction

Gottlieb Haberlandt wanted to study the mutual influence of cells as the smallest "living units" within plant tissue using sterile *in vitro* conditions. Scientists had already shown before him, that whole plants could be generated from isolated embryos, but it was Haberlandt who was able to generate plants from already differentiated tissue by adding plant growth regulators like cytokinins and auxins externally to nutrient media. Today *in vitro* culture of plants is commonly used as a tool in research for plant physiology and breeding, molecular and microbiology and secondary metabolite production. It is a main pillar for the conservation of important culture varieties or endangered species in genebanks, when storage of vegetative plant material is necessary (f. e. *Allium* spp., *Mentha* spp., *Musa* spp.).

In the 1980s it also became an important technology for commercial micropropagation, which made it possible to produce a high number of genetically identical and morphologically homogeneous plants. Sterile conditions eased the production of pathogen-free plants. Another advantage is the possibility to produce independently of seasonal changes in Northern Europe.

In 1963, the International Association of Plant Tissue Culture (IAPTC) was founded as a platform for this fast evolving research field. The major objective of the IAPTC was "to promote the interest of plant tissue culture workers". With increasing fields of application, the association grew to over 4500 members from 123 countries around the world in 1995-1996. After 25 years, it needed to reflect the increasing role and impact of plant biotechnology and was renamed into International Association of Plant Tissue Culture & Biotechnology (IAPTC&B, 1998). Since 2006 it supports as International Association of Plant Biotechnology (IAPB) the plant tissue culture and biotechnology around the world, by publishing the journal "In Vitro Cellular and Developmental Biology – Plant" together with the Society for In Vitro Biology (SIVB) and holding a worldwide recognised conference every fourth year (upcoming conference in South Korea in 2022) (iapbhome.com, 2019).

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In 1985, the Arbeitskreis Deutsche In vitro Kulturen ADIVK (Association of German in vitro culture laboratories) was founded as a network for scientific institutions and commercial laboratories to further study and communicate micropropagation techniques and protocols for new genera. WINKELMANN et al. reported in 2006 about the state-of-the-art of commercial micropropagation in Germany, analysing data of the annual statistics of the commercial ADIVK members, which were collected each year on a voluntary basis, making no claim to be complete. WINKELMANN et al. (2006) focused on the development of the number of companies, plants and plant genera since its foundation in 1985 to 2004.

Ever since then commercial ADIVK members continued to report their annual production numbers, so that after another 15 years we want to highlight the development of the different application fields of *in vitro* culture technology and the development of plant production for that period. We extracted data of WINKELMANN et al. (2006) from the years 1985 and 1995 and continued with the data of 2005 (10 year steps), followed by 2010 and 2015 (5 year steps) and the latest data from 2017. Development of production capacities in other countries will be only marginally touched, where they have an impact on the German market.

Micropropagation as a tool for mass propagation

In vitro culture of plants is established by using all parts of plant tissue (meristems, buds, leaves, stems), though for each plant species the optimal explant type must be investigated. Zygotic embryos can be used to induce somatic embryogenesis. Explants are surface sterilized and transferred to nutrient media containing macro- and microelements, vitamins, plant growth regulators and sugar. For each plant species the composition of the nutrient media must be optimized. The most common nutrient media are modified after MURASHIGE and SKOOG (1962). The usage of the plant growth regulators Cytokinins and Auxins makes controlled shoot or root growth possible. Micropropagation is carried out by successive cutting of shoots, followed by rooting and later hardening of plantlets in greenhouses.

A challenge remains the optimization of the process from establishment of *in vitro* culture to micropropagation at large scale and greenhouse production. Evaluation of these process steps results in a production success, which is calculated as the number of plantlets, that need to be produced *in vitro* to produce one sellable plant. This production process is the clue for cost calculations and can show a large range from sometimes less than 30% to 90% (QUAMBUSCH et al., 2017). Still customers's need for uniform appearance and performance of plants make micropropagation a perfect tool for the production of clonal plant material.

ADIVK commercial members state "mass propagation", "stock plant propagation", "pathogen elimination" and "*in vitro* storage" as the most important applications. Large numbers of identical disease-free plants can be produced, meeting market's and customer's demands to get standardised plants of consistent healthy quality. In the beginning mainly ornamentals and small fruits were produced, with orchids and strawberrys as the most important plant genera resp.

species. During the following decades a diversification of micropropagation techniques opened new fields of applications but still the above mentioned are the most important ones for commercial laboratories (WINKELMANN et al., 2006). During the last 15 years since the first analysis of the ADIVK statistics by WINKELMANN et al. (2006) the market for plant production has undergone major changes that all had an impact on the development of companies and plant production. Aim of this paper is to analyse possible reasons and evaluate the state of the art.

Since the foundation of the ADIVK the number of commercial members has risen from 12 in 1985 to 28 in 1995 and remains more or less stable until to date (27 in 2017). The number of plants reported by these members has risen from 5 million plants per year to an immense increase of 49 million plants in 2005 and dropped down to 28 million in 2017 (Fig. 1).

The statistics of the ADIVK distinguish **different categories of plants** according to their market. For the group of ornamentals the orchids are shown separately due to their outstanding development and high production numbers. Small fruits have a remaining importance as well as woody plants. Micropropagation of agricultural species and vegetables as well as medicinal plants remains a niche application (Fig. 2).

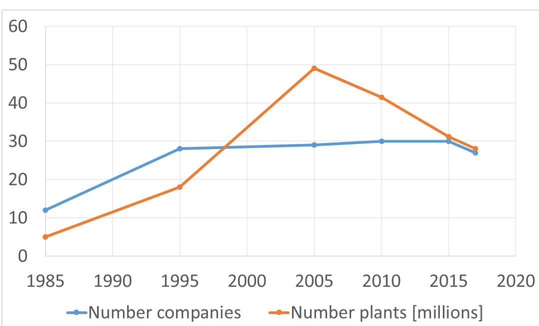

Fig. 1: Plant production via micropropagation in Germany from 1985 to 2017. Number of companies and number of plants (stated by ADIVK members, voluntary basis).

The production of **orchids** is the most important commercial application related to plant numbers (46% or 13 million in 2017). When interpreting the numbers it has to be considered that the leading company of orchid production in Germany does not report its production numbers since 2005, but states an annual production of 50 million plants alone in 2017 (MITSCHKA and BÜCHLER, 2018). The increase in orchid production was realised due to higher market prices in the early 2000s, followed by a decrease due to shifts of production capacities to low labour cost countries, a process which is still going on. Today the market in Germany and Europe is almost saturated, so that companies seek new markets for example in the USA (MITSCHKA and BÜCHLER, 2018). Nevertheless the number of orchids sold in Germany remains high with a market share of 34% of all ornamentals in 2017 (Statista.com), *Phalaenopsis* being the most important genus (95%, followed by *Cypripedium*, *Cambria*, *Oncidium* and *Cattleya* species).

In general the market for all **ornamentals** (cut flowers, indoor flowers and flowers for balcony, beds and borders) is highly fragmented and internationalised with breeders, growers and retailers, who all participate in the added value-chain. Micropropagation laboratories provide mass propagated plant material to gardeners and nurseries, as well as mother stocks to breeders. In Germany production value of ornamentals reached altogether 1.1 billion Euro, produced by more than 3,000 companies in 2017 (statista.com). For comparison the biggest European auctioneer in the Netherlands retailed ornamentals of 4.7 billion Euro in 2017, with decreasing numbers (11.7 billion plants) but increasing prices (Taspo.de). Most ornamentals are produced outside of Europe in Kenia, Ethiopia or Israel and imported back into Europe (MITSCHKA and BÜCHLER, 2018). Germany remains a big market for ornamentals but ADIVK members produce a relatively small share of 5 million ornamentals per year.

Micropropagation of ornamentals underlies not only production capacities but also fashions. During the last decades production numbers of different ornamental species has undergone severe changes that are not only due to shifts to low labour cost countries but also to popularity of plant species and the demand for innovations (Fig. 3).

Micropropagation of ornamentals started with the most popular genera in the 1980s namely *Anthurium*, *Spatiphyllum* and *Gerbera* with a peak in production of those species in 1995 (total number almost

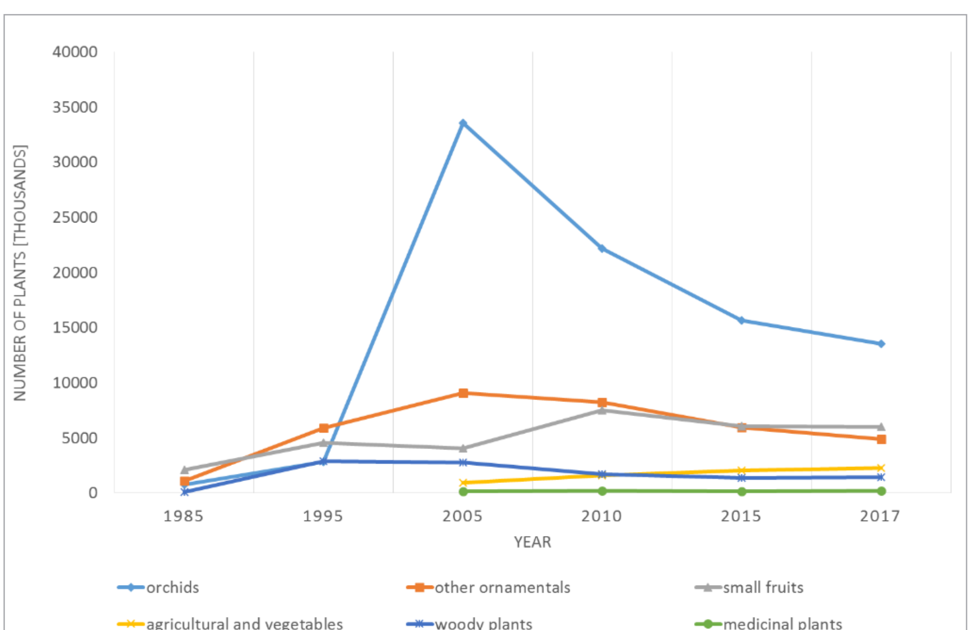

Fig. 2: Plant production numbers of different categories of plants from 1985 to 2017 (stated by ADIVK members, voluntary basis).

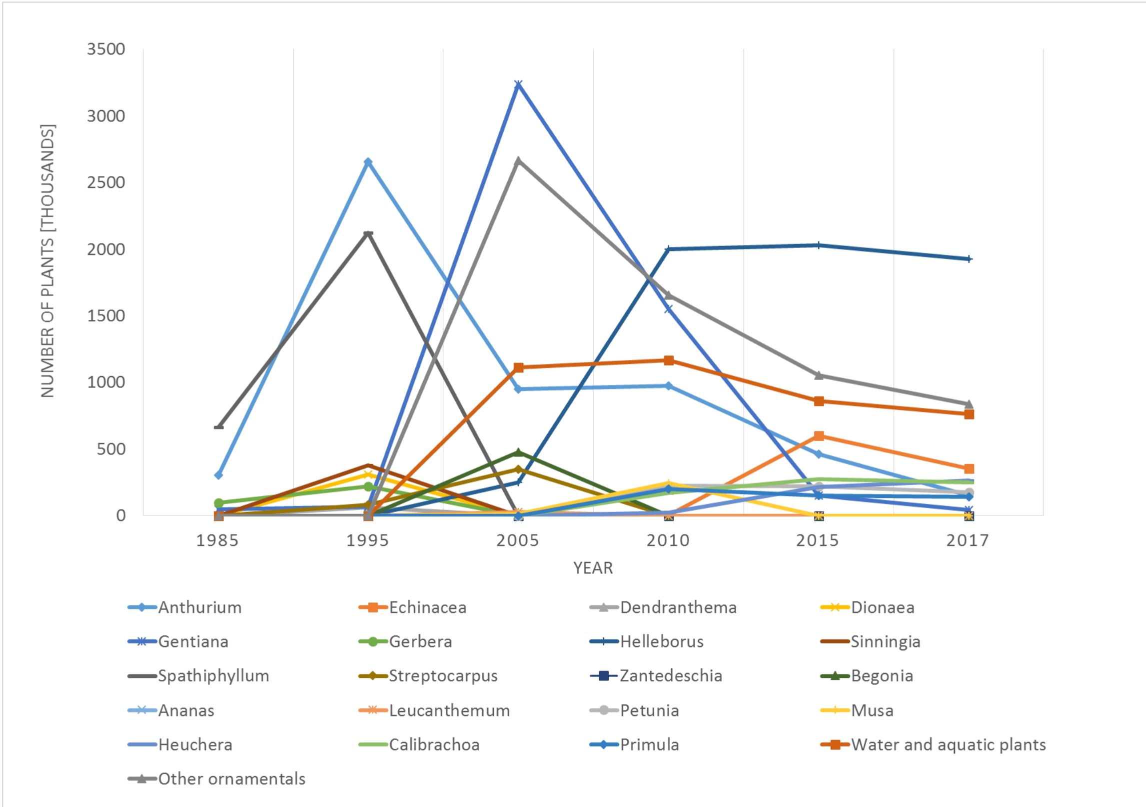

Fig. 3: Production of selected ornamentals from 1985 to 2017 (stated by ADIVK members, voluntary basis).

6 million plants). They were replaced by *Gentiana* and *Dendranthema* until 2005, followed by *Helleborus* which since 2010 shows a stable production. Altogether more than 130 plant genera are reported by ADIVK members, resulting in a share of almost 18% of all plants produced.

The production of **small fruit plants** remains an important share with 6 million plants or 21% of the micropropagation per year (Fig. 4).

Inside the group the highest numbers are generated with *Fragaria* and *Rubus* due to their importance in fruit production. Here the pricing pressure from low labour cost countries resulted in a decrease in *Fragaria* production, but the numbers remain more or less stable since 2015 as there is a demand for disease-free plant material. Since 2010, an increasing number of *Vaccinium* is produced as it is a more and more popular fruit in Germany and growers have a demand for productive, vital plants that produce large and tasty fruits which results in selection efforts (pers. communication).

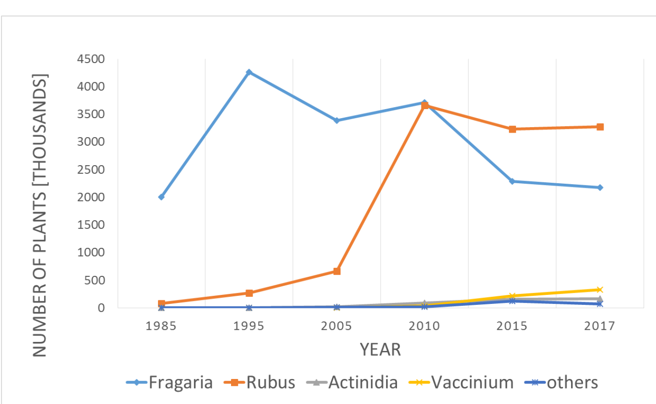

Fig. 4: Plant production numbers of small fruit species from 1985 to 2017 (stated by ADIVK members, voluntary basis).

Woody plants include ornamental shrubs like *Rhododendron*, *Prunus*, *Syringa*, *Rosa* and others and broad leave trees like *Prunus avium*, *Robinia pseudoacacia*, *Betula* spp., *Populus* spp., *Juglans* spp. for forest tree production. In 2017, a total number of 1.44 million plants (5%) was produced (Fig. 5).

For ornamental trees and shrubs *Rhododendron* and *Syringa* have the biggest importance for garden and landscaping. Micropropagation of *Rhododendron* increased from 0 in 1985 to 1.4 million within 10 years. A severe decrease from 2005 to 2010 is mainly due to a shift of production to low labour cost countries especially to Asia. Since then the production is stable with 250,000 plants in 2017. Micropropagation of *Rosa spp*. had its peak in 1995 with 500,000 million plants and is not longer reported today for Germany, whereas *Syringa spp*. shows increasing production numbers with 426,000 per year in 2017. Broad leave tree species for timber production are mainly found within the genera of *Prunus*, *Robinia*, *Betula* and *Populus* but are not distinguished from ornamental shrubs in this figure. Micropropagation of forest trees is estimated to be less than 250,000 plants per year (pers. communication).

High hopes lay on the somatic embryogenesis of conifer trees with many scientific and commercial institutions investigating the optimisation for different conifer tree species, like *Pseudotsuga menziesii* and *Picea abies* for timber production and *Abies nordmanniana* for Christmas tree production in Europe. Only from a Swedish consortium it is known that the production process can be considered as semi-commercial with a production of somatic embryos in Temporary Immersion System, sorting of embryos by camera and mechanical pricking and planting.

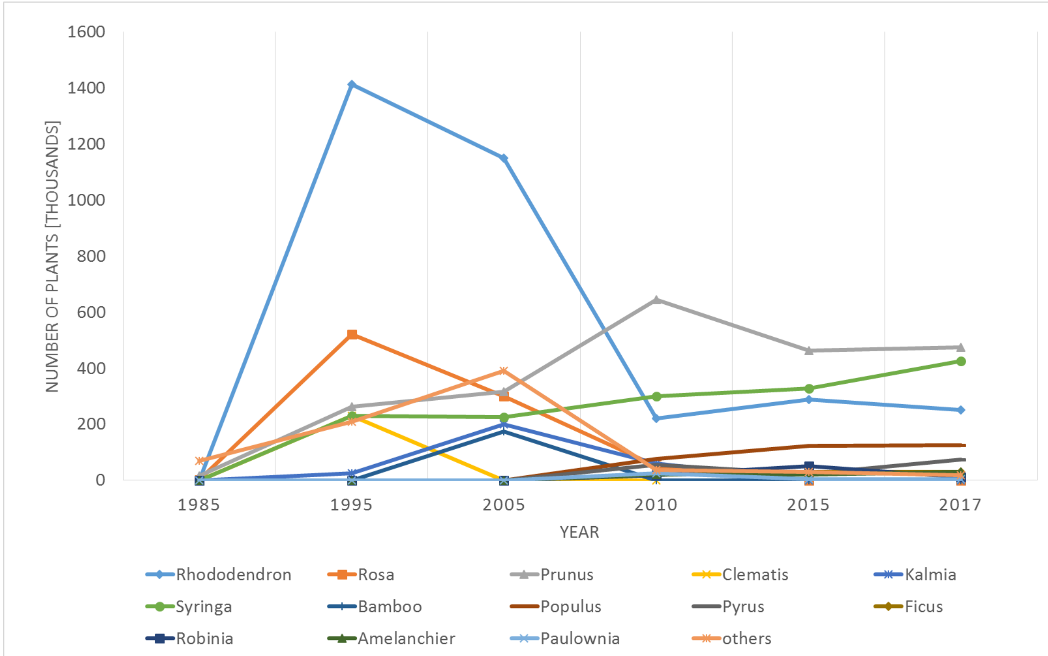

Fig. 5: Plant production numbers of tree species and shrubs from 1985 to 2017 (stated by ADIVK members, voluntary basis).

Agricultural plant species are mainly produced via micropropagation to maintain culture collections, produce virus-free mother stocks and induce double haploids for breeding purposes. Main species are *Solanum tuberosum, Beta vulgaris* and *Hordeum vulgare* each with 0.5 million plants per year which sums up to 2.2 million plants per year for all reported 12 genera (2017).

Micropropagation of **medicinal plants** remains a niche production where *Baptisia tinctoria* (175,000 plants until 2018) makes the biggest share of 92 % of all 15 genera reported.

Other applications

Another application, which was not mentioned above, is the production of **secondary metabolites** via liquid cell cultures. The most prominent example is the production of Taxol® via cell cultures of *Taxus baccata* in aerated stirred tanks. Defined cell lines are upscaled in defined nutrient media and the active ingredient can be extracted and further processed (phytonbiotech.com). Other approaches for the production of Taxol® investigate metabolic pathways of endophytic fungi (Souvik, 2014) or genetically transformed yeasts (Anand, 2013) but without commercial applications until now.

New developments and future perspectives

Micropropagation of plants was also meant as a method to produce sterile plant material, free of bacterial or fungal pathogens. Since the work of Müller and Döring (2009) on bacterial populations in *in vitro* cultures of *Eleutherococcus* spp., investigations on different plant species have shown, that **bacterial** and **fungal endophytes** can live inside plant material, only being detected by molecular techniques (Quambusch et al., 2014; Quambusch and Winkelmann, 2018). These bacteria and fungi must not be phytopathogenic but can stay latent in the plant material without causing any diseases or even function as beneficial endophytes that provide nutrients to plants. The most commonly used examples are symbionts like *Rhizobia* sp. or mycorrhizal fungi. As a consequence, the European Union funded the COST action "Endophytes in Biotechnology and Agriculture" (FA1103, 2011-2015) to let scientists discuss the new challenges and perspectives of these findings and the possibilities of the use of endophytes to create a beneficial microbiome for breeding purposes. It was followed by COST action FA1405 "Using three-way interactions between plants, microbes and arthropods to enhance crop protection and production" which takes additionally the influence of arthropods into account. All these studies and future applications are not possible without micropropagation techniques.

Discussion

Today micropropagation of plant material is a methodology that does not seem to bear any more secrets. Developed techniques provide reliable tools, which are used worldwide. What are the future challenges? We see different intersections that influence a forecast for developments in commercial application and research (Fig. 6).

Intersections differ for companies, who take micropropagation as a reliable method in their production chain and scientists (commercial and institutional), who use it as a tool for their research. Commercial micropropagation laboratories often originate from family-owned or owner-managed nurseries, which saw the advantage to implement the new technology into their production to produce large numbers of homogeneous, pathogen-free plants. Other applications followed with a continuous fragmentation and internationalisation of the market. Today about 60% of ADIVK members are still family-owned or owner-managed.

Family-owned companies see their advantage in a sustainable entrepreneurship with high flexibility, individual personnel management, regional bonds, and innovative power. They shine with exceptional services, personal interactions with their customers and in niches. As weaknesses, they mention unsettled succession plans with potential conflicts between family members and hindered access to capital resulting in less vigour. Next generations will struggle with the balancing act between pricing pressure, the need for innovation and the shortage of skilled personnel (Müller, 2012). In the following we want to highlight a few of the intersections specified in Fig. 6.
For companies the reduction of labour costs to meet pricing pressure remains the most important issue. Winkelmann et al. (2006)

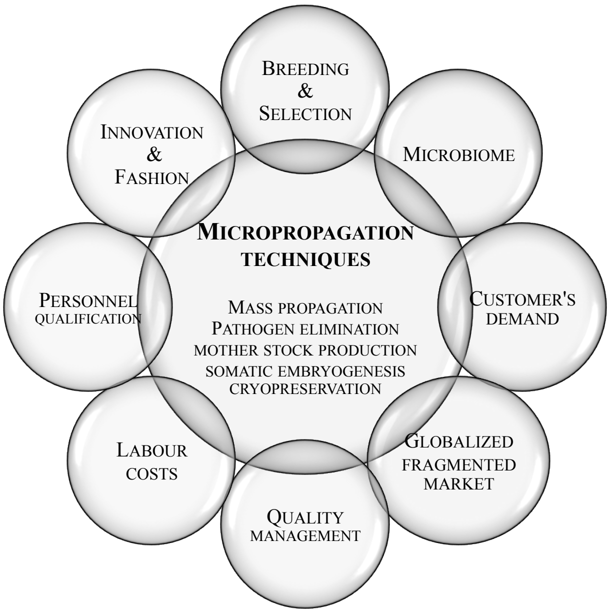

Fig. 6: Micropropagation of plants: intersections in research and commercial application.

had discussed, that new technological innovations such as temporary immersion system (TIS) were needed to reduce labour costs. This forecast did not come true: TIS is not used in commercial plant production to a large extent due to problems with hyperhydricity and contaminations in larger vessels. Reduction of labour costs is mainly achieved by shifting manual labour to countries with lower salary level like East Europe, Africa, Asia or India. But when we report here a shift of production capacities to low labour cost countries it does not necessarily mean, that companies from those regions took over market shares but, that German companies also founded sister companies or built strategic partnerships with companies from abroad. This enables them to install quality standards that still meet their customer's demands, but the shift of production numbers from Germany to those partners is not shown in the annual statistics due to ADIVK's regulations.

The highly fragmented and internationalised market for ornamentals poses additional challenges for companies. As soon as a market is saturated, as it seems to be for orchids in Europe, they must explore and capture new markets, with the risk of failure. Germany's largest orchid producer expanded with production facilities to the United States to hold and increase his market share of impressing 25%. Today development of new micropropagation techniques is less important than logistics and interaction with customers. For example, barcoding of culture vessels as a safety system for calculation of propagation cycles, tracking of vessels or online analysis of stock numbers guarantees the utmost reliability of the production. An intensive quality management assures customers that mother stocks are virus-free, clonal plants are not mixed and that each partner of the added value-chain is aware of the standardised process steps he is responsible for (MITSCHKA and BLÜCHER, 2018).

WINKELMANN et al. (2006) also proposed that new protocols for the propagation of woody plants should be developed as there seemed to be a willingness of foresters to pay higher prices. More protocols have been developed but still foresters are not easily convinced to invest more capital in optimised plant material. The development of protocols only seems to be economically interesting for rare and highly valuable tree species such as wavy grain acer or *Juglans* hybrids. High expectations lay on the development of fully mechanised production systems for conifer trees like Norway spruce or Douglas fir via somatic embryogenesis. Thus, until now those efforts still need investment in technological innovations to produce trees in profitable numbers.

For agricultural crops and vegetables, the application of *in vitro* culture cannot be a methodology for mass propagation as the added value is too low in comparison to seeds, but it is an important tool in research and breeding to optimise specific characteristics. A new challenge is the breeding of beneficial endophytic microbiomes for agricultural crops. Still a lot of research is needed in this field which results in two conferences to be held in 2019: the Austrian Symposium "MiCROPe: Microbe-Assisted Crop Production – Opportunities, challenges & Needs" and the 2nd Eucarpia Workshop on Implementing Plant-Microbe Interactions in Plant Breeding (Eucarpia, European Association for research on plant breeding).

Conclusions

Micropropagation of plant material is no longer an own research field but will remain as a technique for companies to produce a large number of plants for the international market and at the same time as an irreplaceable tool in breeding and research. The different plant groups have different challenges for micropropagation companies. Still the stable number of companies since 1995 shows that they can persist in a more and more globalised market finding their application field or market niche. What will guarantee their future is an innovative production chain in combination with breeding and selection for new fashionable varieties (orchids, ornamentals), an enhanced quality (woody plants, small fruit plants, medicinal plants) or improved microbiome (agricultural crops and vegetables).

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